R01 Grant Ideas

Presented by Dan Scoles on Tuesday 9-18-2012

Title

SCA2 and calcium dysfunction

Investigating the underlying common molecular defects of SCA2 and related cerebellar ataxias

Molecular targets for SCA2 and other ataxias defined by investigation of common molecular features.

Discovering common molecular features of SCA2 and related cerebellar ataxias as therapeutic targets.

Common molecular features of SCA2 and related cerebellar ataxias as therapeutic targets.

Investigating calSCA2fication.

Background

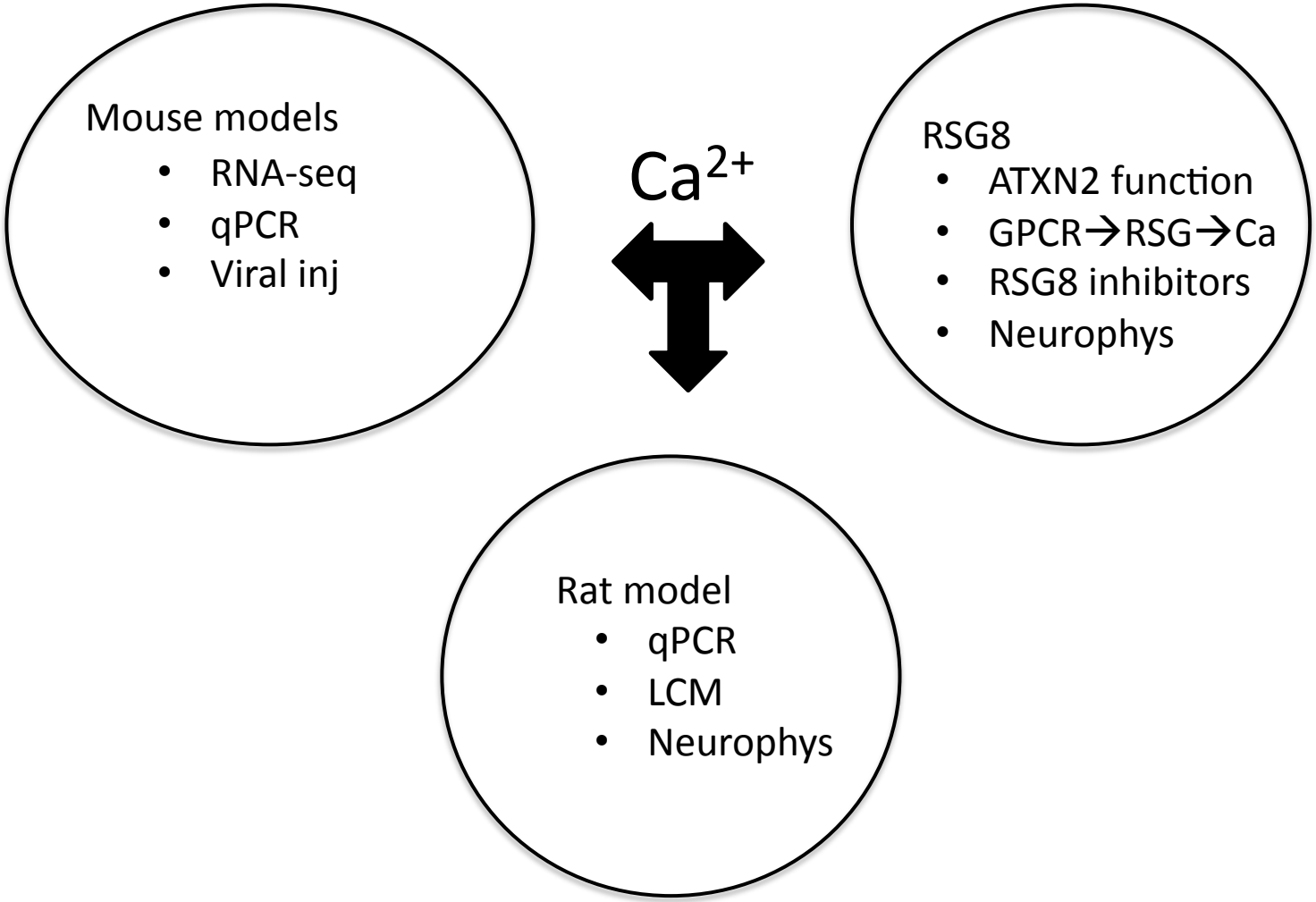
- SCA2 and other SCAs are associated with abnormalities in cellular calcium leading to defective neuronal function.
- For SCA2 we have observed abnormal high abundances of cytoplasmic calcium associated with aberrant polyQ expanded ataxin-2 interaction with IP3R receptor. We have pinpointed the associated neurophysiological defect as reduced Purkinje cell firing rate coincident with the onset of SCA2 motor phenotype of *Pcp2-ATXN2* mice.
- Additionally, we have established a molecular endophenotype for *Pcp2-ATXN2* mice as well as for a new series of *ATXN2*-BAC mouse lines with varying CAG repeat lengths. This molecular endophenotype is comprised of the quantitatively defined expression levels of multiple key cerebellar and Purkinje cell-specific genes which become reduced as SCA2 motor phenotype onsets and PCs are compromised.
- Among the nine genes that make up this ataxia endophenotype we have noted that *Rgs8* is radically lowered, to levels that are significantly lower than the Purkinje cell genes *Pcp2* and *Calb1*. *RGS8* is an attenuator of Ca^{2+} channel inhibition, and collectively this suggests a functional link between *Rgs8* and the SCA2 motor phenotype onset.
- We have also recently discovered that mutation in the *Atp2c3* calcium channel gene is responsible for the ataxia phenotype in the Long Evans shaker rat, and *ATP2C3* mutation was subsequently found responsible for human congenital cerebellar ataxia. This key finding reinforces the common link between calcium channels and ataxia and provides a valuable rat model for our proposed studies.

Hypothesis

The overall hypothesis of this study is that abnormal calcium homeostasis is a common feature of cerebellar ataxias, and is caused by dysfunctional calcium ion channels.

The combined investigation of calcium systems, molecular and motor phenotypes, and neurophysiology, applied to our mouse and rat models of ataxia, will lead to the identification of common features of ataxia that can be exploited therapeutically for SCA2 and other related ataxia disorders.

Parts of the R01



Specific Aims

1) Identifying common molecular features among SCA2 mouse models
    - RNA-seq
    - Validation by qPCR

2) Rgs8 and *ATXN2* function
    - Can Rgs8 complement *ATXN2-Q127* function?
    - What is the effect of RGS8 small molecule inhibitors?
    - Evaluate all the Ca channels RGS proteins regulate in context of SCA2??

        N-type R-type

        Neurophysiology in SCA2 mice with and without RGS8 inhibitor

3) Molecular and neurophysiological features of Purkinje cells in the LE shaker rat.
    - Test by qPCR if any changes in aim 1 show up in the rat
    - Evaluate localized changes
        - LCM→ qPCR
        - Physiology
    - Ratorod?
    - ΔG/R plot

Tom' Grant

Tom Otis wants to further study the mGluR1 → IP3R1 system in Purkinje cells making use of the channelrhodopsins.

Channelrhodopsin (ChR2)

- Activation of channelrhodopsin in neurons by blue light depolarizes neurons by passive ion movement. This activates neurons. (Influx of Na+ and Ca2+ is associated with action potential firing)

Halorhodopsin (NpHR)

- Activation of Halorhodopsin in neurons by green/yellow light actively pumps chloride ions into the cell and silences firing. The cells become hyperpolarized inhibiting action potentials.

Tsubota et al., 2011. Optogenetic Manipulation of Cerebellar Purkinje Cell Activity In Vivo. PlosOne:6(8):e22400.

Tom wants us to complement his grant

- Behavioral analysis
- LCM in same region Myria records from.
    - Tom said that he thought that cerebellar regions of greatest PC loss might be characterized by greater mGluR1 expression. ??